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NUTRITION STUDIES WITH THE SULFONAMIDES

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NUTRITION STUDIES WITH THE SULFONAMIDES

The development of the sulfonamides as bacteriostatic agents has been a major step forward in the biological sciences. Their role in medicine and in pharmacology is well recognized. Of equal significance are the outstanding contributions to research in microbiology, biochemistry and nutrition which have been made possible through the discovery and use of these compounds. The intertwining of these three fields through the common factor of the sulfonamides is a complex, but fascinating story which has led to the evolution of new and important ideas in modern nutrition.

The basis for the use of the sulfonamides in nutrition studies lies in the early studies leading up to the concept of vitamin biosynthesis by intestinal microorganisms.* With the dual realization that such microorganisms can be a significant source of supply of essential vitamins to the host animal and that bacteriostatic agents such as the sulfonamides can effectively interfere with this source of supply, an entire new field of nutrition investigation was revealed.

Early Studies with Sulfonamides:

The use of sulfonamides as research tools in nutrition studies followed the introduction in 1940 by Marshall and co-workers (1) of a new sulfa derivative, sulfaguanidine. Sulfaguanidine, because it is poorly absorbed, was announced to be a highly effective bacteriostatic agent for intestinal organisms. This announcement was received with great interest by nutritionists and biochemists who visualized the possibilities of employing the chemical as a means of producing and subsequently studying specific vitamin deficiencies and requirements in experimental animals. Sulfaguanidine made possible also the study of the extent of vitamin synthesis. It afforded, further, opportunities for the study of the requirements of a vitamin on varying dietary regimes by abolishing, where possible, the interfering factor of intestinal synthesis. The use of the insoluble sulfonamides has permitted a clearer study and understanding of the complex metabolic interrelationships between vitamins and has been the means of conclusively demonstrating that certain vitamins actually are synthesized in the intestinal tract.

**cf. Borden's Review of Nutrition Research,* May, 1946—"The Biosynthesis of Vitamins."

Sulfa-Induced Deficiencies:

The incorporation of various sulfonamides into purified animal diets precipitates a wide variety of serious physiologic and anatomic disturbances (3). The pathologies thus produced, diverse though they are in nature, have almost all been traced to interference with the biosynthesis of essential metabolites, particularly the vitamins.* (Table 1.) Many of the sulfa-induced lesions have centered about the blood, involving disturbances in the clotting mechanism, abnormalities of blood cell formation and of the bone marrow. Disturbances of the thyroid and kidney and degenerative changes in heart muscle, blood vessels and liver cells have been reported also, but these have not been tied up clearly with nutritional dysfunctions.

Vitamin K Deficiency: The first reported use of sulfaguanidine in nutrition experiments was made a year after Marshall's announcement by Black, McKibben and Elvehjem (4). They found that rats receiving the drug at a level of 0.5 per cent in a purified diet soon failed to grow. The inhibition of growth could be corrected by administering either liver extract or *p*-aminobenzoic acid. Growth response was immediate following administration of liver extract and somewhat delayed following *p*-aminobenzoic acid. This was explained by the assumption that the drug had interfered with the bacterial synthesis of certain unknown growth factors which were supplied immediately by the liver extract and which were made available indirectly and thus more slowly after *p*-aminobenzoic acid had permitted restoration of normal intestinal synthesis.

In this first published study of the inhibitory effect of sulfaguanidine on the synthesis of nutritional factors by intestinal bacteria, Black *et al* noted no disturbance other than an interference with growth processes in animals on a sulfaguanidine-containing diet. Because, at that time, vitamin K was known to be one of the vitamins supplied to the rat by the intestinal flora, evidence of a disturbance in the blood-clotting mechanism was sought.† No delay, however, was observed in the clotting time of the blood of animals even after seven weeks on the sulfaguanidine diet. In a later experiment, however, the problem was further investigated by determinations of the clotting time of diluted plasma prothrombin, since plasma prothrombin levels can be reduced considerably before evidence of delayed clotting time of whole blood can be detected. As had been anticipated, the incorporation

*Other metabolites, such as the amino acids, recently have been assumed to be involved in the biological interactions between sulfonamides and the intestinal flora (2).

†*cf. Borden's Review of Nutrition Research,* April, 1943—"The Story of Vitamin K."

Table 1. Sulfonamide Induced Lesions in Experimental Animals and Vitamins Implicated

Vitamin Implicated	Lesions
(Used successfully in prophylactic or therapeutic experiments)	
K	Increase in prothrombin time of plasma Hemorrhages Increase in clotting time of whole blood
Biotin	Dermatitis and alopecia
Pantothenic acid	Hemorrhagic necrosis of adrenals Alopecia Porphyrin-staining of fur and whiskers
E	Hyaline necrosis and calcification of voluntary muscles
L. casei factor	Leukopenia Granulocytopenia Anemia Hypocellularity of bone-marrow Achromotrichia Porphyrin-caked whiskers
Inositol	Alopecia
Unidentified	Hyaline sclerosis and calcification of blood vessels Necrosis of heart muscle Cardiac aneurysm Liver damage
No vitamin involved	Hypertrophy and hyperemia of thyroid Kidney damage

—from Daft and Sebrell (3).

of sulfaguanidine not only inhibited growth, but now was found to cause a marked drop in the normal prothrombin level of the blood *(hypoprothrombinemia)* accompanied by an increase in prothrombin clotting time from an average of 39 seconds to an average of 113 seconds (5). Liver extract or *p*-aminobenzoic acid, both of which had been reported to counteract the growth-inhibiting effect of the drug (4), were both likewise effective in counteracting the blood-clotting disturbance. Vitamin K was found to be effective in restoring the prothrombin level to normal, but had no effect on growth. The unknown factor in liver responsible for counteracting the sulfaguanidine-induced growth inhibition and hypoprothrombinemia was found to be distinct from either *p*-aminobenzoic acid or vitamin K, but was thought to possess properties similar to those of "folic acid," or the *L. casei* factor. While Black *et al* were seeking an explanation for the mechanisms involved in these reactions, a new drug—sulfasuxidine (succinylsulfathiazole)—was made available for similar experiments (6). Black *et al* found it to have effects similar to those of sulfaguanidine which were counteracted by liver extract and vitamin K. Strangely enough, however, in experiments by Wright (6), *p*-aminobenzoic acid was unable to reverse either the growth inhibition or vitamin K deficiency induced by the new sulfa derivative.

In the meantime, Daft, Ashburn and Sebrell (7), while investigating the production of biotin deficiency in sulfonamide-fed rats, reported the occurrence of extensive hemorrhages in these animals. Later these were shown by Kornberg, Daft and Sebrell (8) to be manifestations of vitamin K deficiency and were found to respond to vitamin K therapy but not to treatment with biotin or folic acid either singly or in combination. Kornberg *et al* found that the severity of the hemorrhagic deficiency produced varied with the particular sulfonamide used;* that the type of dietary casein, depending on its vitamin K content, also influenced the development of the deficiency and that regardless of the drug used to induce the deficiency, uniform responses were obtained to orally administered vitamin K.

At that time, although the mechanisms involved in the antagonisms between the sulfonamides and the vitamins were in an early stage of conjecture, a definite contribution already had been made to the science of nutrition: A vitamin deficiency disease (in this case, of vitamin K) difficult to produce by dietary means alone could be produced uniformly and easily by the feeding of sulfonamides and thereupon subjected to further investigation.

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*Sulfapyrazine, sulfadiazine and sulfathiazole produced severest hemorrhages; sulfaguanidine, sulfanilamide and succinylsulfathiazole were much less effective.

Deficiencies of the B Vitamins: The early successes with sulfa-induced vitamin K deficiencies stimulated the further use of these drugs in experimental diets. It was hoped that the drugs would reduce the number of intestinal organisms, that the synthesis of vitamins by such organisms would be decreased correspondingly and that this ultimately would lead to the production of new or more severe vitamin deficiencies. The production of such vitamin deficiencies has been achieved successfully, but some doubt remains as to whether they have been brought about by so simple a mechanism as that postulated.

The most important studies with the B vitamins have centered around the proof obtained through the use of sulfonamides, that biotin and folic acid are synthesized in the intestinal tract of the rat. That *E. coli* and other bacteria of the intestinal tract are capable of synthesizing biotin *in vitro* was demonstrated by Landy and Dicken (9) and Burkholder and McVeigh (10), while similar evidence for the *in vitro* synthesis of folic acid by intestinal bacteria was obtained by Thompson (11). Then, in 1942, several independent groups of investigators observed serious growth disturbances and severe blood dyscrasias (involving abnormalities of the various white blood cells and of the bone marrow) in rats fed sulfaguanidine and sulfasuxidine in purified diets (12, 13). The use of liver extracts rich in "folic acid" concentrates was found by a number of research groups to be extremely effective in treating these disorders (13, 14, 15, 16). That the cure was definitely attributable to "folic acid" was demonstrated unequivocally later when Daft and Sebrell (17) repeated these experiments with the newly isolated crystalline "folic acid" *(L. casei* factor) factors of Pfiffner (18) and of Stokstad (19). As in the case of vitamin K deficiency, it was shown by Kornberg *et al* (20) that the blood disorders of "folic acid" deficiency could be induced without the use of sulfa drugs by dietary means alone, but to a more limited extent.

The growth failure of rats on these sulfa-containing purified diets, at first treated successfully with liver extract, soon was demonstrated to respond specifically to "folic acid" and biotin. Merely withdrawing the sulfonamides did not produce an immediate growth response, a fact which was taken to indicate that bacterial synthesis in the intestinal tract had been supplying the animals with the necessary vitamins and that this synthesizing mechanism had been interfered with by the addition of the sulfonamides. These experiments, leading to the discovery of the role and biosynthesis of folic acid and biotin have further suggested the existence of possible new vitamin factors,

normally synthesized by the intestinal flora and, therefore, as yet unsuspected as nutritional factors. The further use of the sulfonamides along the lines of these experiments is expected to lead to the uncovering of such as yet unidentified nutrients.

The induction of deficiency syndromes which have responded to treatment with inositol (21) and to pantothenic acid directly (22) has been reported as a result of incorporating sulfasuxidine and sulfapyridine, respectively, into purified animal diets. A number of disorders occurring on sulfa diets characterized by degenerative changes in heart muscle, blood vessels, and liver cells has been listed (3) but these have not been traced to any vitamin deficiency. Because these changes have not been observed in rats receiving liver extracts does suggest, however, that a dietary deficiency may be involved.

Vitamin E Deficiency: The incorporation of sulfonamides into experimental diets has resulted in symptoms strongly suggestive of vitamin E deficiency (3). These observations seem at first to be somewhat inexplicable on the basis of the theory of sulfonamide inhibition of intestinal synthesis, because there is no evidence that vitamin E can be synthesized by intestinal microorganisms. A possible explanation may exist in the recent announcement of Milhorat and Bartels (23) that the biologically effective form of vitamin E is a compound with inositol. Since presumptive evidence exists for the intestinal synthesis of inositol and the inhibition of such synthesis by sulfasuxidine (21), it is conceivable that such inhibition might lead indirectly to vitamin E deficiency symptoms through the destruction of the inositol portion of the vitamin E-inositol complex.

Vitamin Interrelationships:

An interesting by-path in the study of B vitamin requirements and synthesis with the aid of sulfonamides, is the demonstration that certain of the B vitamins are so intimately interrelated that a deficiency of one may precipitate a deficiency of one or more of the others, thereby leading to the development of a highly complex deficiency syndrone. For example, Daft *et al* (24) recently have reported that symptoms of anemia, granulocytopenia and bone marrow hypoplasia (various forms of blood cell deficiency) have been observed in animals on a pantothenic-acid deficient diet, but not in animals receiving dietary pantothenic acid. Nevertheless, the administration of pantothenic acid to the deficient animals failed to alleviate the blood disorders! The white cell disturbance responded to folic acid therapy; the anemia

responded only to a combination of folic acid and pantothenic acid. The blood dyscrasias, which were present to varying extents and in various combinations in the different animals, seemed to be the result of a simultaneous deficiency of two vitamins which might not have occurred had either one of the vitamins concerned been adequately supplied.

Wright and Welch (25) observed further interesting phenomena along these lines. They found that feeding rats a highly purified diet containing sulfasuxidine caused a marked reduction in the liver stores of folic acid, biotin and pantothenic acid, with symptoms of pantothenic acid deficiency. The storage of other B vitamins was unaffected. The drop in the liver stores of pantothenic acid was particularly remarkable because this vitamin was being provided amply in the diet. Even more strange was the fact that added supplements of pantothenic acid failed to counteract the symptoms of pantothenic acid deficiency and had no effect on the severely reduced amount of this vitamin in the liver. Administration of biotin and folic acid, however, caused both a prompt recovery from symptoms of pantothenic acid deficiency (graying of hair and discoloration of whiskers by red porphyrin deposits), as well as a restoration of the pantothenic acid level of the liver to normal. The explanation for these unexpected occurrences is not yet at hand. Welch and Wright discuss two possibilities: First, that folic acid and biotin may play a role in the bacterial synthesis of pantothenic acid or second, that folic acid and biotin may be essential somehow for the proper storage and utilization of pantothenic acid. Martin (26), on the other hand, who also observed symptoms of achromotrichia (graying of hair) in rats receiving sulfaguanidine or sulfasuxidine and adequate pantothenic acid, concluded that this, and other symptoms, probably are not specific signs of pantothenic acid deficiency.

Conclusion:

The position of the sulfonamides in biological research has been admirably summed up in the following statement by Daft and Sebrell (3):

"The significance of the results of these investigations *(with the sulfonamides)* cannot be properly evaluated as yet but we know they have proved of interest to workers in several fields. To the clinician they have suggested that some of the so-called toxic effects of the sulfonamides may have a dietary origin or aspect and that they may be amenable to treatment, either prophylactically or therapeutically, with specific nutrients. To the pharmacologist they have reemphasized the importance of diet in studies of the toxicology

and pharmacology of drugs. In the field of bacteriology they have reawakened interest in what we may from the teleological point of view call the function of the intestinal bacteria or more broadly, in the influence of these bacteria for weal or woe on the host animal. To the investigator in the field of nutrition the use of drugs, particularly of the sulfonamides, in experimental diets has proved a most valuable tool. From the list of signs which have developed in animals receiving these drugs and which are as yet unascribable to a deficiency of any known vitamin it appears that the future for this type of experimentation holds much promise."

REFERENCES CITED

1. MARSHALL, E. K., JR., A. C. BRATTON, H. J. WHITE and J. T. LITCHFIELD. Sulfanilyguanidine: chemotherapeutic agent for intestinal infections. Bull. Johns Hopkins Hosp. *67,* 163 (1940).

2. MARTIN, G. J. Mixture of pure amino acids as substitute for dietary protein. Proc. Soc. Exp. Biol. Med. *55,* 182 (1944).

3. DAFT, F. S. and W. H. SEBRELL. Sulfonamides and vitamin deficiencies. Vitamins and Hormones. *3,* 49 (1945).

4. BLACK, S., J. M. MCKIBBIN and C. A. ELVEHJEM. Use of sulfonamides in nutrition experiments. Proc. Soc. Exp. Biol. Med. *47,* 308 (1941).

5. BLACK, S., R. S. OVERMAN, C. A. ELVEHJEM and K. P. LINK. The effect of sulfaguanidine on rat growth and plasma prothrombin. J. Biol. Chem. *145,* 137 (1942).

6. WELCH, A. D. Feder. Proc. *1,* 171 (1942).

7. DAFT, F. S., L. L. ASHBURN and W. H. SEBRELL. Biotin deficiency and other changes in rats given sulfanilylguanidine and succinylsulfathiazole in purified diets. Science *96,* 321 (1942).

8. KORNBERG, A., F. S. DAFT and W. H. SEBRELL. Production of vitamin K deficiency in rats by various sulfonamides. P. S. Pub. Health Rpts. *59,* 832 (1944).

9. LANDY, M. and D. M. DICKEN. Biotin synthesis by microorganisms. Proc. Soc. Exp. Biol. Med. *46,* 449 (1941).

10. BURKHOLDER, P. R. and I. MCVEIGH. Synthesis of vitamins by intestinal bacteria. Proc. Nat'l Acad. Sci. *28,* 285 (1942).

11. THOMPSON, R. C. Univ. Texas Pub. No. *4237,* 87 (1942).

12. WELCH, A. D., P. A. MATTIS and A. R. LATVEN. Toxicological study of succinylsulfathiazole. J. Pharnacol. Exp. Therap. *75,* 231 (1942).

13. SPICER, S. S., F. S. DAFT, W. H. SEBRELL and L. L. ASHBURN. Prevention and treatment of agranulocytosis and leukopenia in rats given sulfanilylguanidine or succinylsulfathiazole in purified diets. U. S. Pub. Health Rpts. *57,* 1559 (1942).

14. AXELROD, A. E., P. GROSS, M. D. BOSSE and K. F. SWINGLE. Treatment of leucopenia and granulopenia in rats receiving sulfaguanidine in purified diets. J. Biol. Chem. *148,* 721 (1943).

15. RANSONE, B. and C. A. ELVEHJEM. Value of biotin, folic acid concentrates and liver extract in diet of rats fed succinylsulfathiazole. J. Biol. Chem. *151,* 109 (1943).

16. KORNBERG, A., F. S. DAFT and W. H. SEBRELL. Production and treatment of granulocytopenia and anemai in rats fed sulfonamides in purified diets. Science *98,* 20 (1943).

17. DAFT, F. S. and W. H. SEBRELL. Successful treatment of granulocytopenia and leukopenia in rats with crystalline folic acid. U. S. Public Health Rpts. *58,* 1542 (1943).

18. PFIFFNER, J. J. and others. Isolation of antianemia factor (vitamin B*c*) in crystalline form from liver. Science *97,* 404 (1943).

19. STOKSTAD, E. L. R. Some properties of a growth factor for Lactobacillus casei. J. Biol. Chem. *149,* 573 (1943).

20. KORNBERG, A., F. S. DAFT and W. H. SEBRELL. Dietary granulocytopenia in rats corrected by crystalline L. casei factor ("folic acid"). Proc. Soc. Exp. Biol. Med. *58,* 46 (1945).

21. NIELSEN, E. and A. BLACK. Role of inositol in alopecia of rats fed sulfasuxidine. Proc. Soc. Exp. Biol. Med. *55,* 14 (1944).

22. WEST, H. D., N. C. JEFFERSON and R. E. RIVERA. Effect of aromatic hydrocarbons on growth. Reversible inhibition of pantothenic acid by sulfapyridine. J. Nutr. *25,* 471 (1943).

23. MILHORAT, A. T. and W. E. BARTELS. Defect in utilization of tocopherol in progressive muscular dystrophy. Science *101,* 93 (1945).

24. DAFT, F. S., A. KORNBERG, L. L. ASHBURN and W. H. SEBRELL. Anemia and granulocytopenia in rats fed a diet low in pantothenic acid.

25. WRIGHT, L. D. and A. D. WELCH. The role of folic acid and biotin in the utilization of pantothenic acid by the rat. Science *97,* 426 (1943).

26. MARTIN, G. J. Folic acid in nutritional achromotrichia. Proc. Soc. Exp. Biol. Med. *51,* 353 (1942).

NEWS DIGEST

VITAMIN LOSS IN COOKED EGGS—Eggs from hens routinely fed a high riboflavin diet were analyzed for riboflavin and thiamin. Analysis of 32 raw and 32 prepared eggs showed an average cooking loss of 7.7 per cent for riboflavin and 15.0 per cent for thiamin. Poached eggs showed an appreciably higher loss of riboflavin than boiled, fried or scrambled, probably because of the loss of some egg white in the cooking water. Scrambled eggs lost the least riboflavin. Since all of the thiamin of the egg is in the yolk, this may explain why thiamin is not lost to a greater extent in poaching as compared to other methods of cooking. Thiamin loss was quite uniform with all methods of preparation. *J. Am. Diet. Assoc. 22,* 315 (1946).

MUSHROOM PROTEIN—Recent experimental studies have indicated that the protein of mushrooms is of high quality, capable of supporting growth and sustaining life in experimental animals (when fed as the sole source of protein in the diet). Although it has been established that mushroom protein contains all the essential amino acids, they are present in lower concentrations than in the milk product casein or in the proteins of soybean meal. The percentage of protein in fresh mushrooms has been calculated to be over 2½ per cent, a quantity which compare favorably with that of many fresh vegetables. *Jr. Am. Dietetic Assoc. 22,* 318 (1946).

DIET AND LIVER DISORDER — Twenty patients with cirrhosis of the liver were treated with a high protein, low fat diet supplemented with yeast, choline and the amino acid, cystine. Twelve of these patients with livers which were not enlarged showed no response to the therapy, whereas seven of the eight patients with large livers made a good recovery. A comparison of the treated group of large-livered patients with a similar group of fifteen patients who were treated with only a high protein, low fat diet plus yeast, indicated that the combination of choline and cystine had a favorable influence in the treatment of cirrhosis. The striking difference in the response of patients with large livers compared to patients with unenlarged livers suggests that the combination of choline and cystine is effective where fatty changes in the liver are suspected. This bears out earlier experimental animal studies on the lipotropic (antifat) effects of choline.* *J. Am. Med. Assoc. 130,* 190 (1946).

AMINO ACIDS AND CORNEAL WOUNDS—Studies on the effect of topically applied amino acid mixtures to wounds of the cornea indicate that a new and effective means of promoting healing of such wounds, particularly where ulceration is involved, soon may be at hand. The rationale of the treatment is based on the nutritional processes peculiar to corneal tissue. The cornea, because it is a non-vascular tissue, is unique in that it receives its nourishment by diffusion of fluids through its entire substance. Injury to the cornea may disturb this diffusion leading to vascularization (abnormal infiltration of blood vessels) and loss of transparency. Some years ago the successful use of amino acids, particularly cystine, to stimulate tissue culture growth *in vitro* had been reported. Recently this led to an attempt to influence the regeneration of experimental wounds of the cornea by administration of amino acids to the tissue while still in the living body. Various types of incisions were made across both corneas of a group of experimental animals. The amino acids were applied as a solution by dropper or as an ointment. The amino acid solu-

*cf. *Borden's Review of Nutrition Research,* "Labile Methyl Groups in Nutrition," October, 1942.

tion was dropped into the right eyes of the animals, while the left control eyes received a corresponding amount of physiological saline solution. Boric acid ointment was used in conjunction with the amino acid ointment in the treated eyes and alone in the controls. A complete regeneration of the corneal defect in the eyes treated with amino acids was achieved in 12 to 42 hours (average, less than 30 hours), while in the control eyes the healing process required 55 to 120 hours. The amino acid ointment has been used in conjunction with other measures in over 100 as yet unreported clinical cases with man. It is pointed out that although it is difficult to evaluate the data quantitatively because other therapy was included, it nonetheless appears that the application of amino acids accelerates the healing of corneal wounds particularly when ulcerative processes are involved. *Proc. Soc. Exp. Biol. Med. 61,* 165 (1946).